Fig. 1A

Fig. 1A, continued

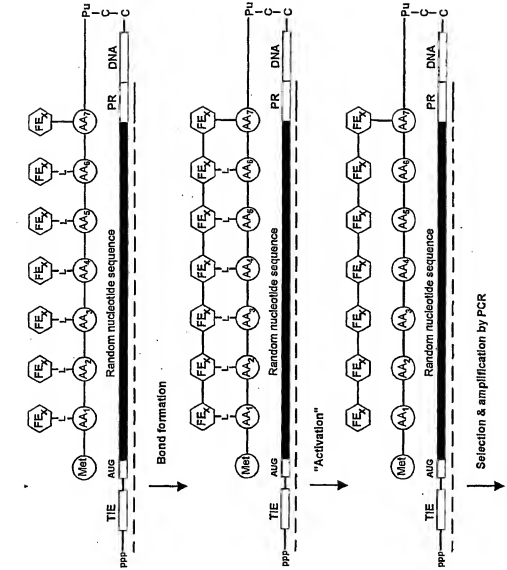


Fig. 1B

Templated branched molecules - the principle
dsDNA
T7-pro
TIE
ATG
Random nucleotide sequence
PR
RNA synthesis and purification
mRNA
ppp
AUG
3'-end addition of DNA-puromycin tag
DNA
C-C-Pu
Translation (I. e. PURE) FE-AA charged tRNAs
Coupling of peptide product to puromycin moiety
Sc
Met
Pu
C

Fig. 1B, continued

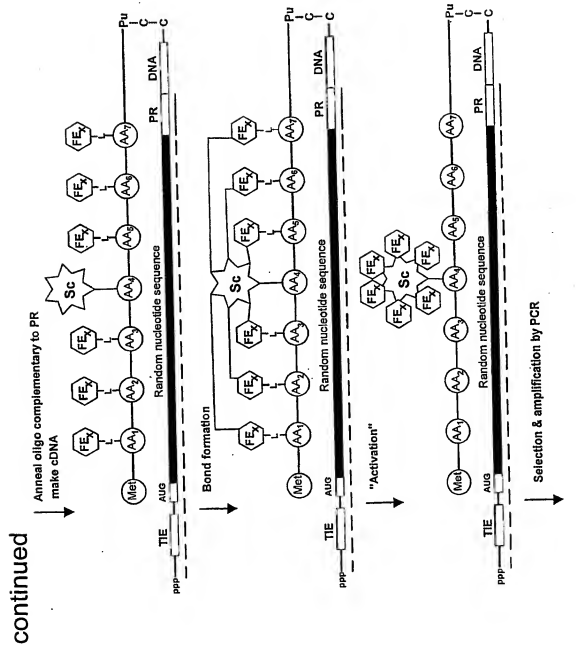


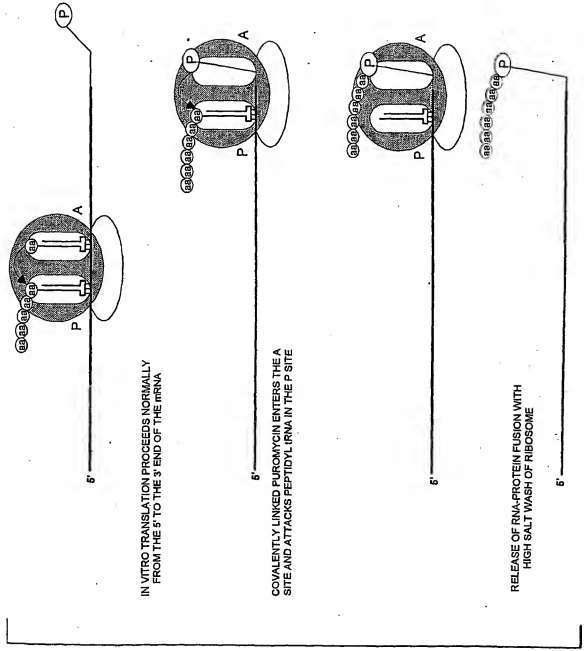
Fig. 1C

Display of Functional Entities on a Peptide Backbone

Fig. 2

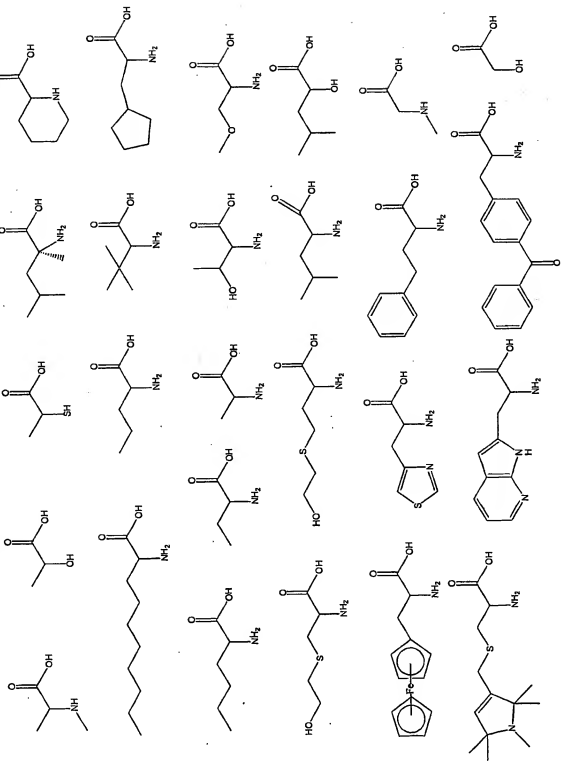
PROFusion
A
T7 PROMOTER
TE
AUG
RANDOMIZED REGION
3' CONSTANT REGION
B
5'
PUROMYCIN-TETHERED OLIGO IS LIGATED TO mRNA(GENERATED FROM ABOVE CONSTRUCT) IN THE PRESENCE OF A SPLINT AND DNA LIGASE
+
DNA SPLINT
DNA LIGASE
P
5'
NOTE:FOR SHORT ORFS, THIS WHOLE TEMPLATE CAN BE MADE SYNTHETICALLY

Fig. 2, continued



C

Fig. 3 Non-standard- and pseudo amino acids incorporated onto peptides by ribosome mediated translation.



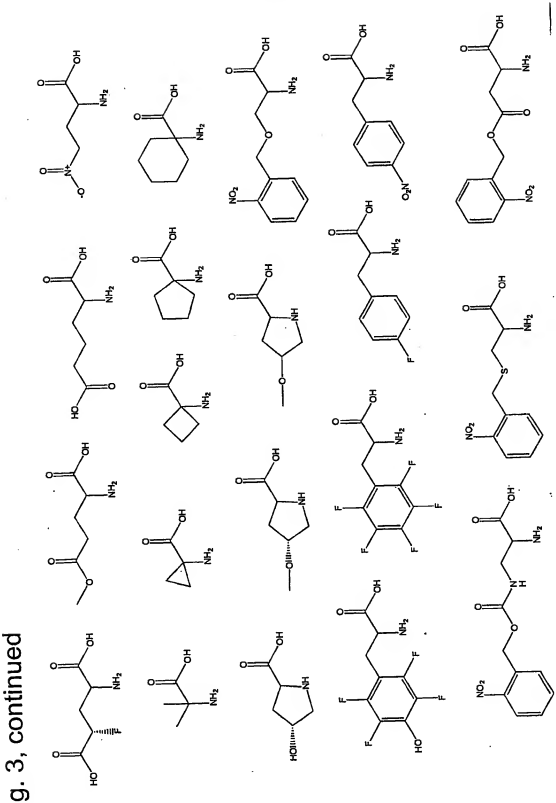


Fig. 3, continued

Fig. 4A **Example of a first building block**

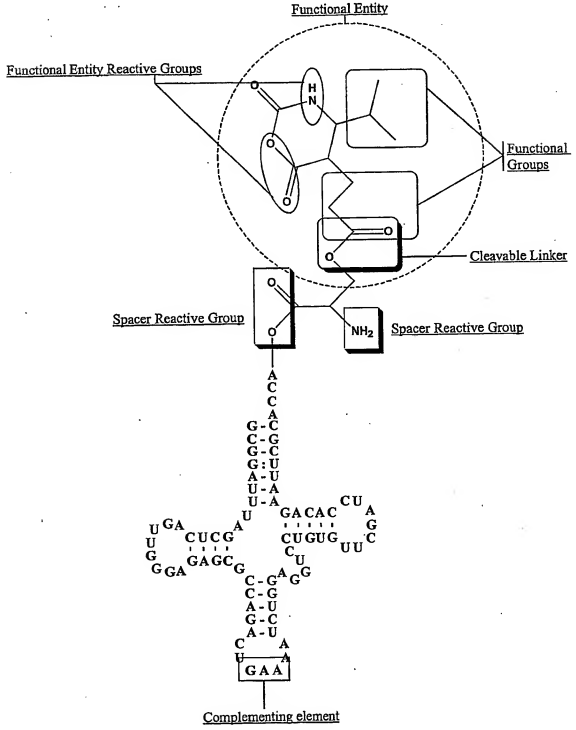


Fig. 4B

Example of a second building block

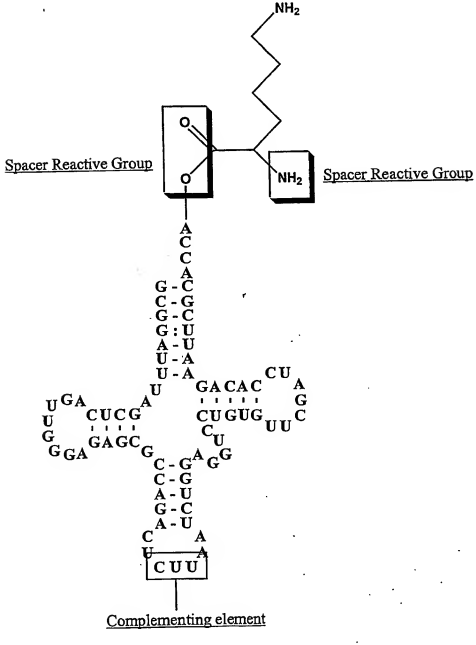
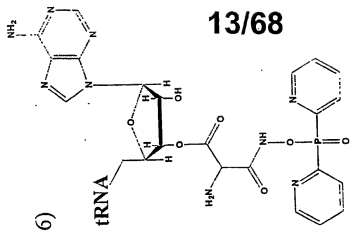
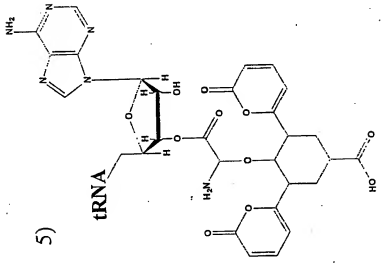


Fig. 4C

Examples of tRNAs charged with FE-AA units





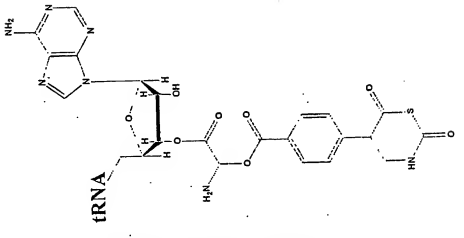
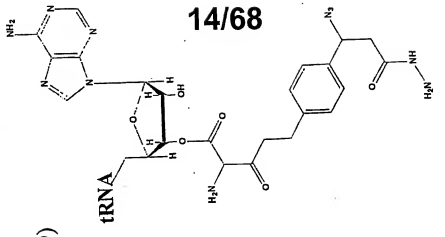
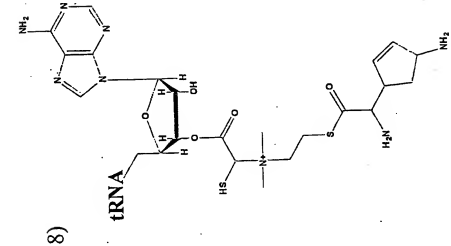
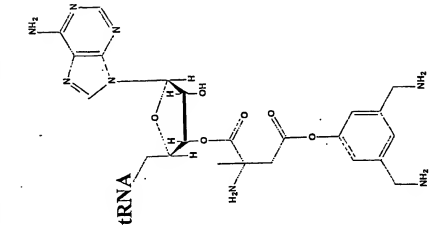


Fig. 4C, continued

Fig. 4C, continued







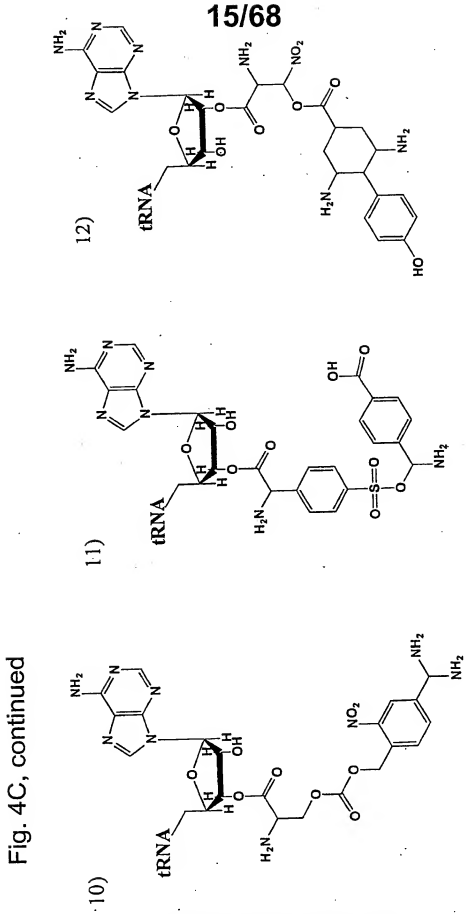
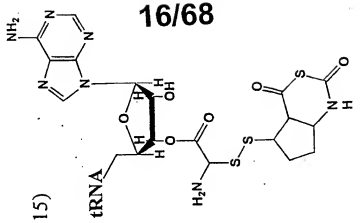
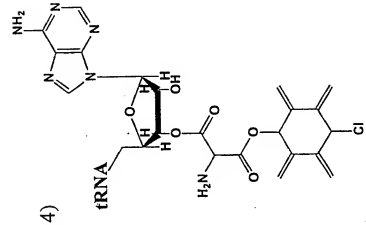


Fig. 4C, continued





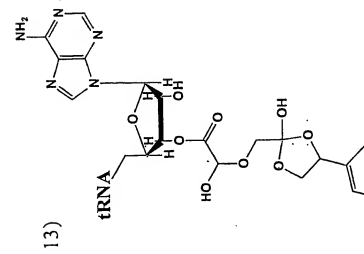
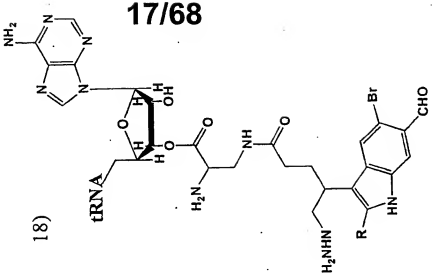
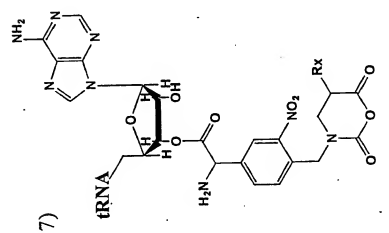


Fig. 4C, continued





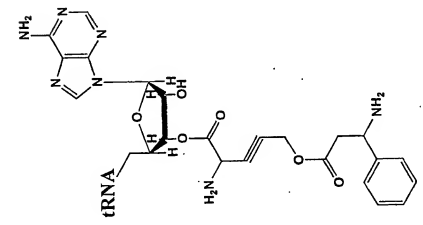


Fig. 4C, continued

Fig. 5A

Enzymatic charging of tRNAs catalysed by amino acid tRNA synthetases

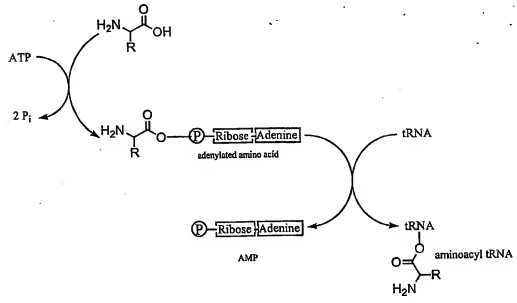


Fig. 5B

Chemical aminoacylation of tRNAs

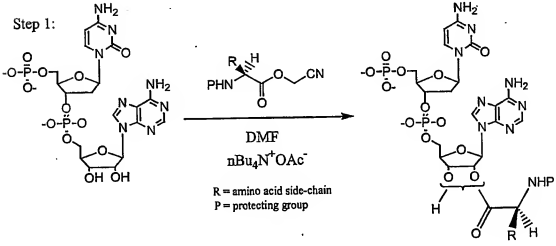


Fig. 6

Bond formation between functional entities and activation of the templated molecule

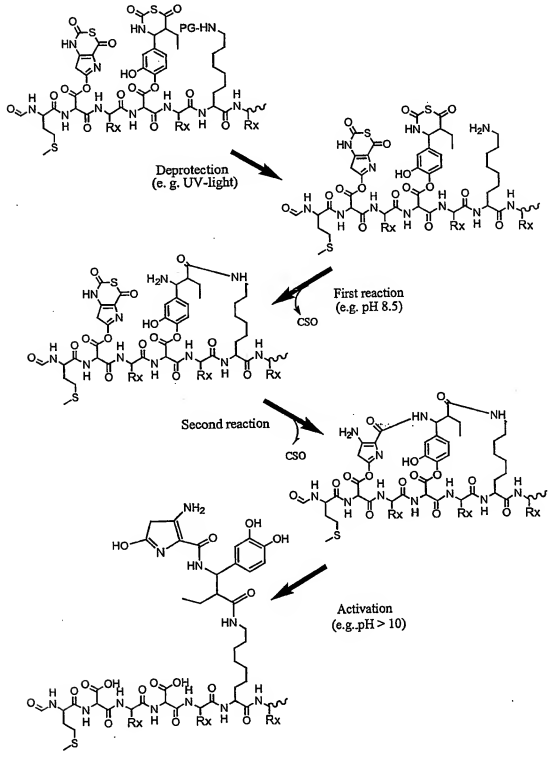


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Fig. 7

alpha-helix display of functional entities

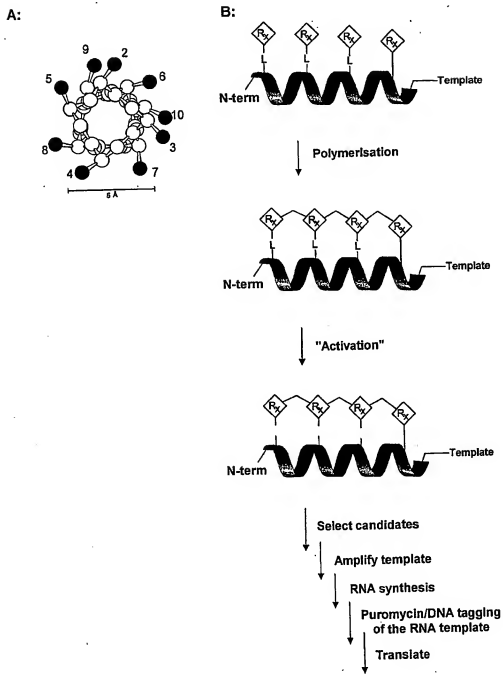

Fig. 8

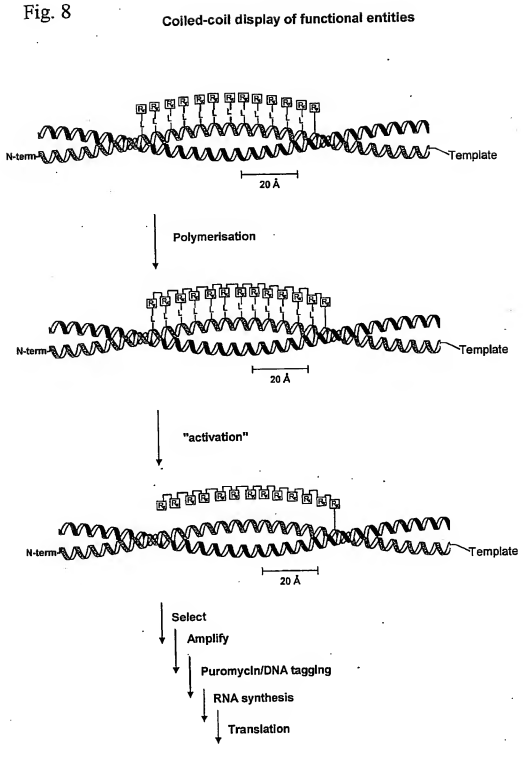
Coiled-coil display of functional entities
N-term
Template
20 Å
Polymerisation
N-term
Template
20 Å
"activation"
N-term
Template
20 Å
Select
Amplify
Puromycin/DNA tagging
RNA synthesis
Translation

Fig. 9

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. Display of functional entities by a collagen-like triple helix structure

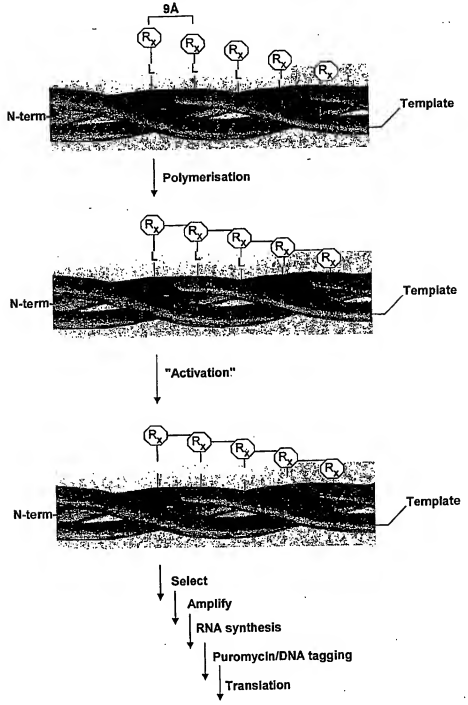
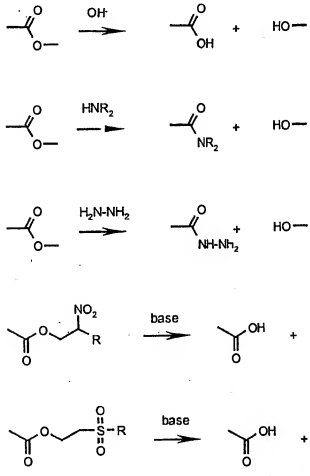


Fig. 10

Cleavable linkers and protection groups, cleaving agents and cleavage products.

A. Base (nucleophilic) cleavage.



B. Photocleavage

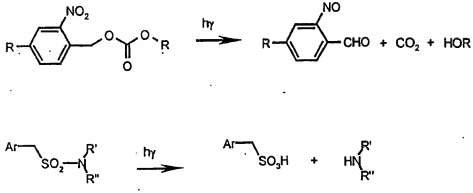
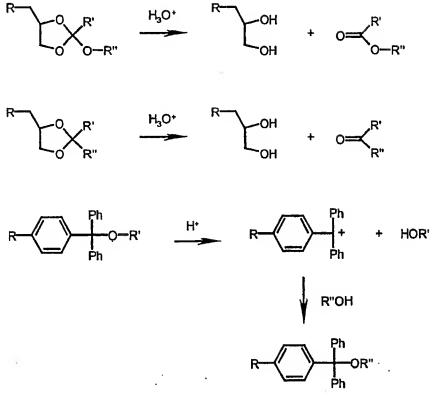


Fig. 10, continued

C. Acid cleavage



D. Catalytic cleavage.

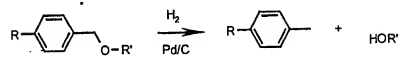
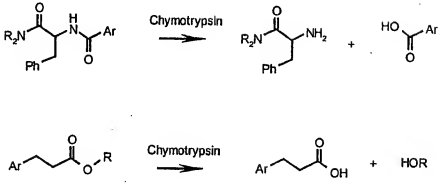
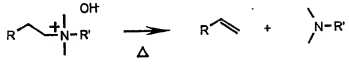


Fig. 10, continued

E. Enzymatic cleavage.



F. Cleavage by temperature increase.



G. Miscellaneous

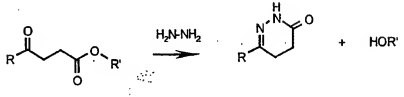


Fig. 11

Polymerization by reaction between neighboring reactive groups.

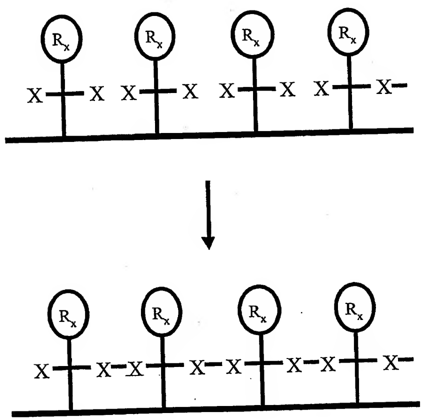


Fig. 11, continued

Ex. 1. Coumarin-based polymerization

Fig 12. Polymerization between neighboring non-identical reactive groups.

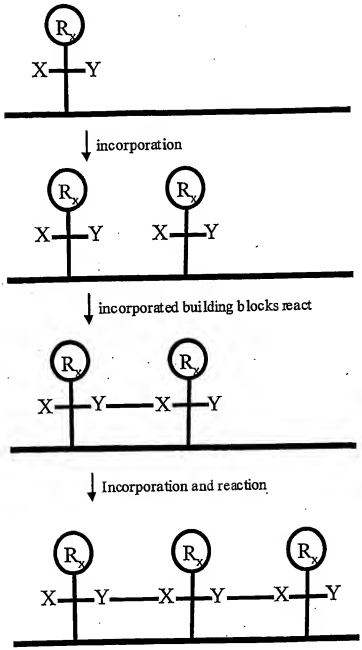


Fig. 13. Cluster formation in the absence of directional polymerization.

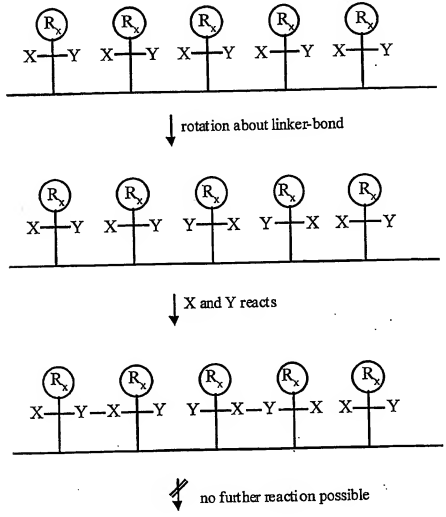


Fig 14. Zipping-polymerization and simultaneous activation.

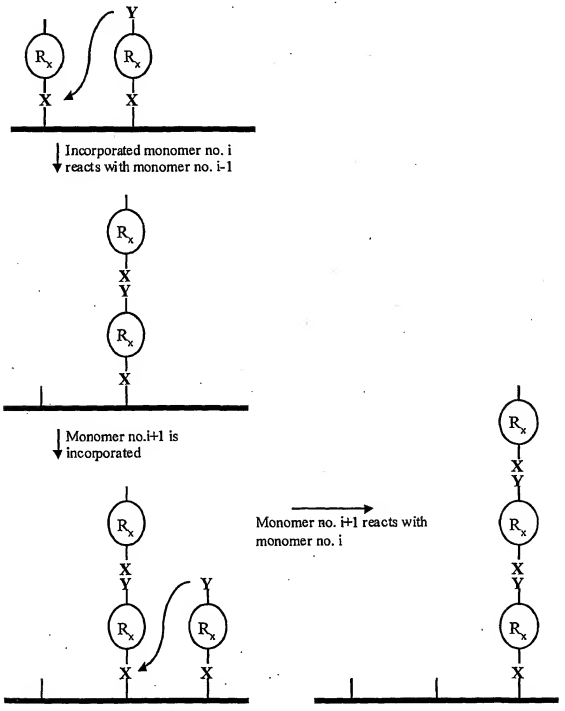


Fig. 14, continued 31/68

Example 1. Polymerization and activation (thioesters)

A.

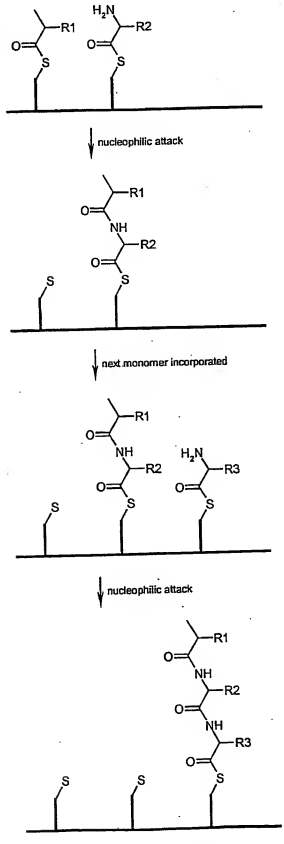


Fig. 14, continued

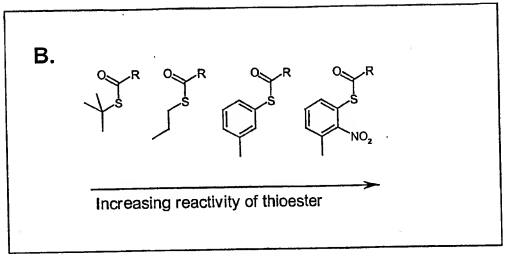


Fig. 14, continued

Example 2. Polyamine formation and activation

Fig. 15

"Fill-in" polymerization (symmetric XX monomers).

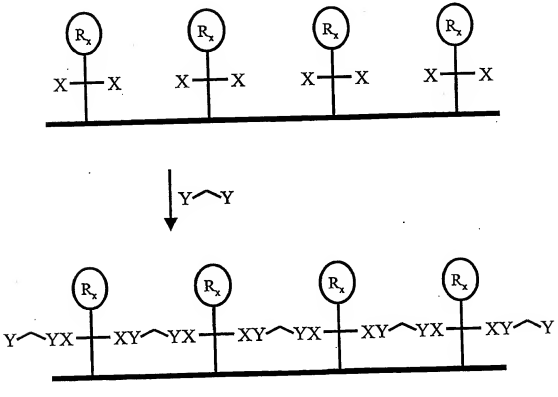
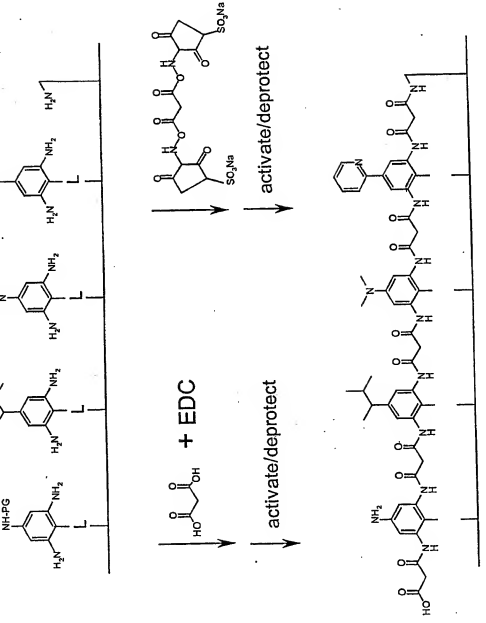


Fig. 15, continued

Example 1. Poly-imine formation by fill-in polymerization

Fig. 15, continued

Example 2. Polyamide formation



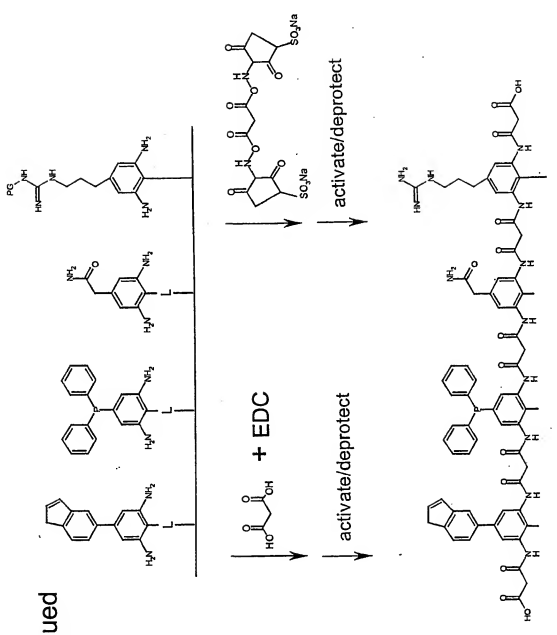


Fig. 15, continued

Fig. 15, continued

Example 3. Polyurea formation

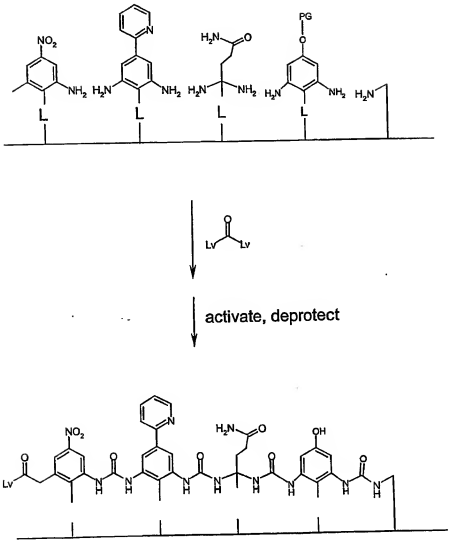


Fig. 15, continued **39/68**

Example 4. Chiral and achiral polyamide backbone formation

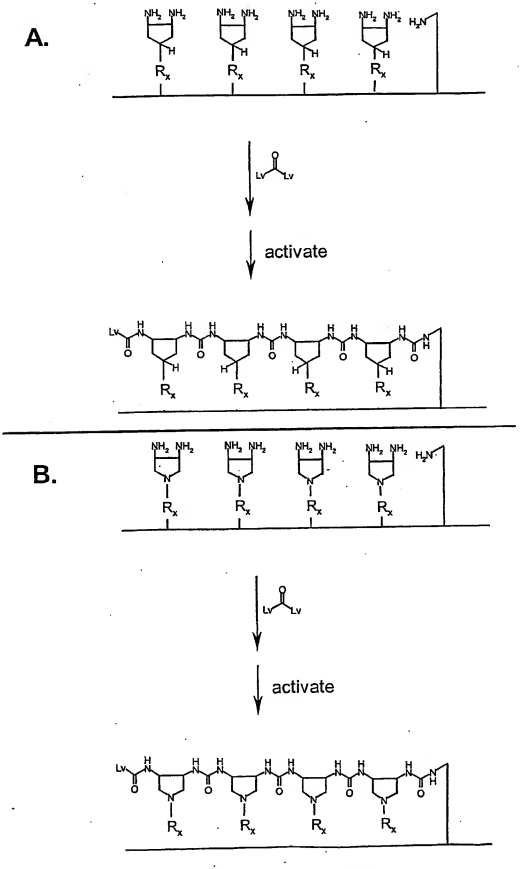


Fig. 15, continued

Example 5. Polyphosphodiester formation

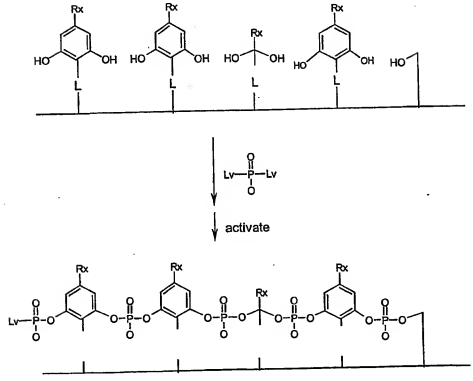


Fig. 15, continued

Example 6. Polyphosphodiester formation with one reactive group in each monomer building

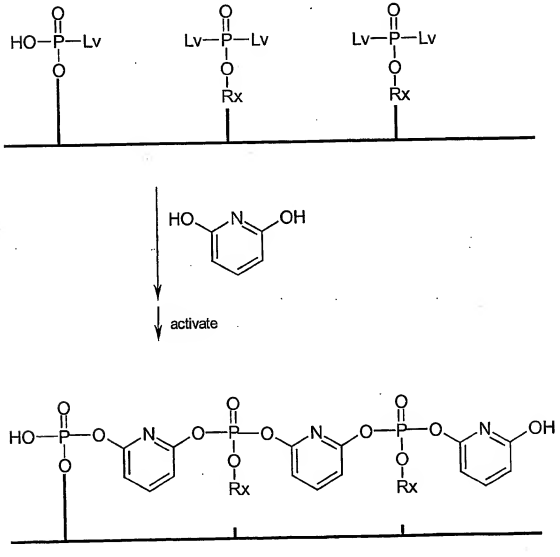


Fig. 15, continued

Example 7. Pericyclic, "fill-in" polymerization

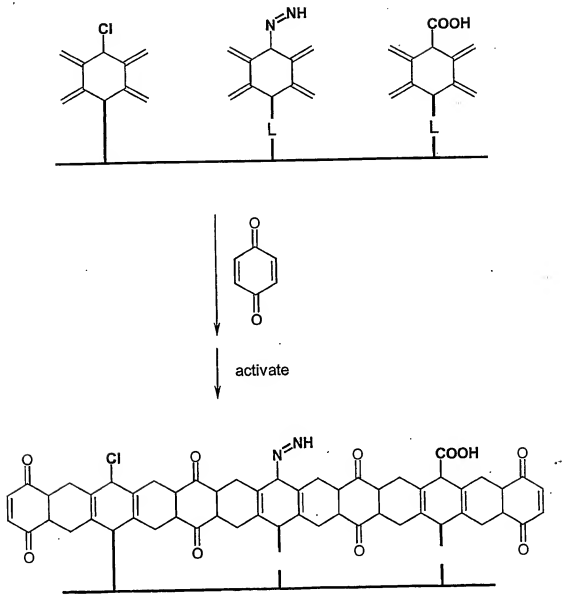


Fig. 16. "Fill-in" polymerization (asymmetric XS monomers).

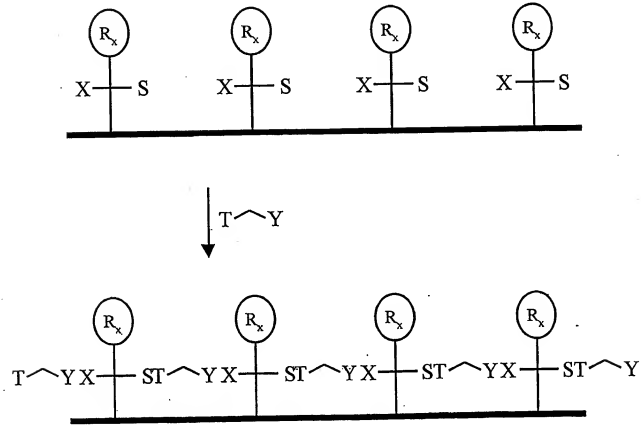


Fig. 16, continued

Example 1. Fill-in polymerization by ketone-hydrazide reaction and by modified Staudinger ligation

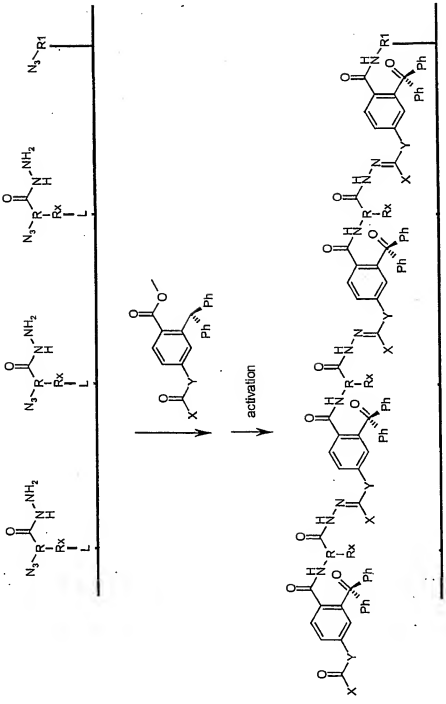


Fig. 17

"Zipping" polymerization

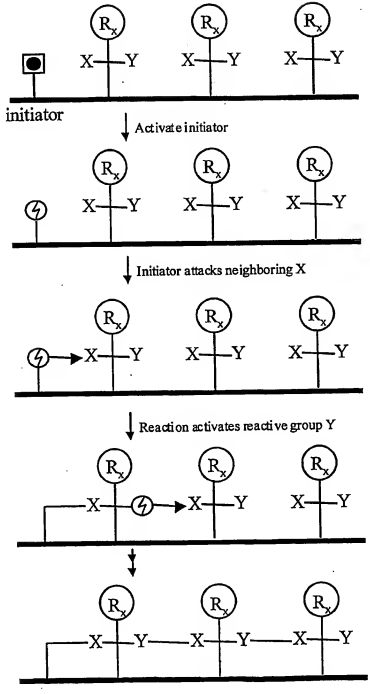


Fig. 17, continued

Example 1. Radical polymerization

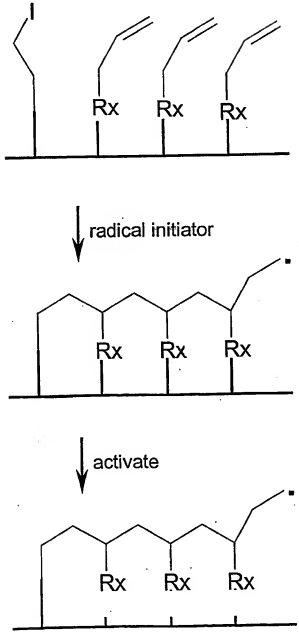


Fig. 17, continued. Example 2. Cationic polymerization

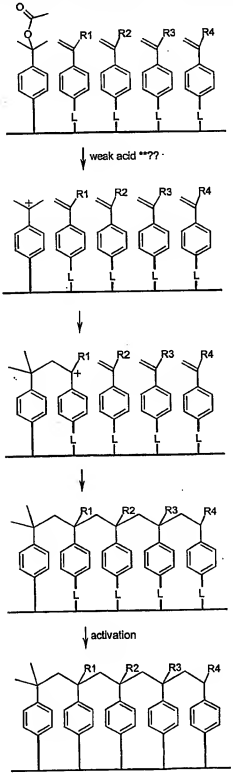


Fig. 18. Zipping polymerization by ring opening.

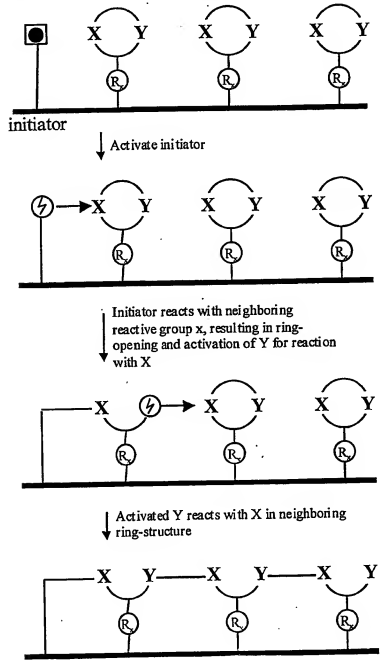


Fig. 18, continued. Example 1.

"Zipping" polymerization of N-thiocarboxyanhydrides, to form ß-peptides.

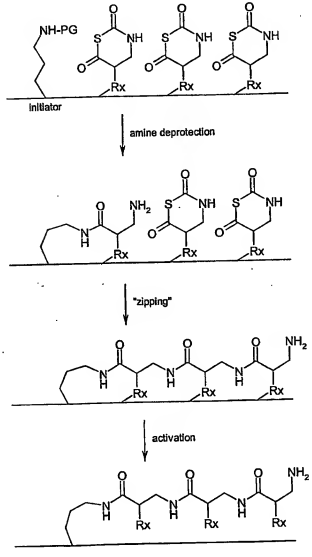


Fig. 18, continued. Example 2. "Zipping" polymerization of 2,2-diphenylthiazinanone units to form ß-peptides.

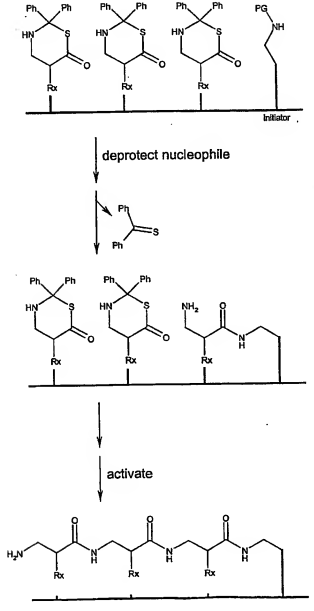


Fig. 18, continued. Example 3. Polyether formation by ring-opening polymerization.

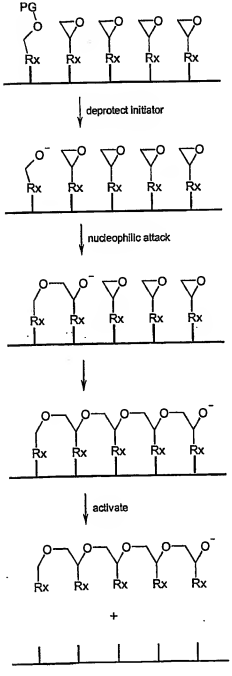


Fig. 19

Zipping-polymerization and activation by rearrangement.

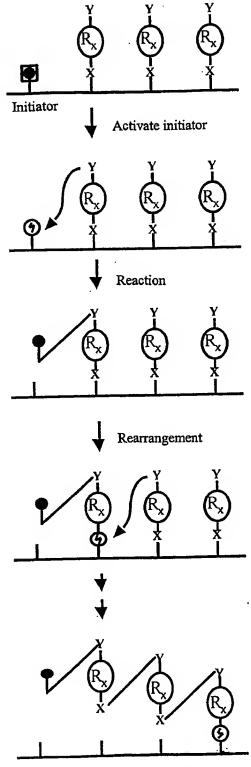


Fig. 20. Zipping-polymerization and activation by ring opening.

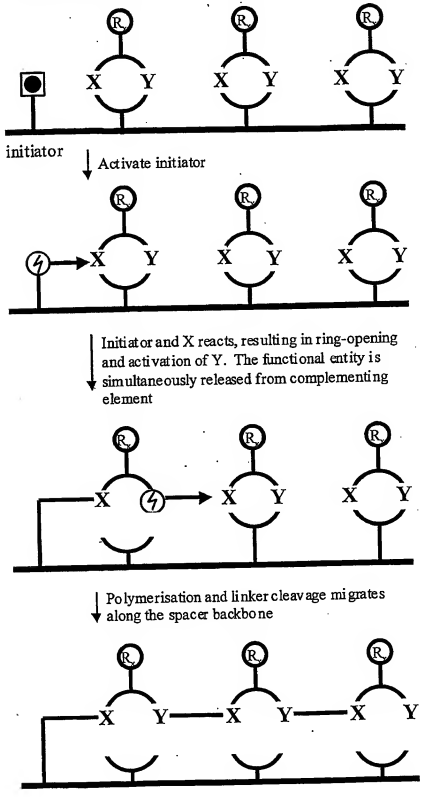


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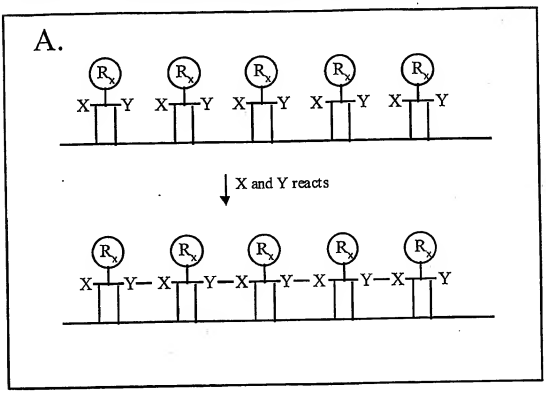
Fig. 21.
Directional polymer formation using fixed functional units.



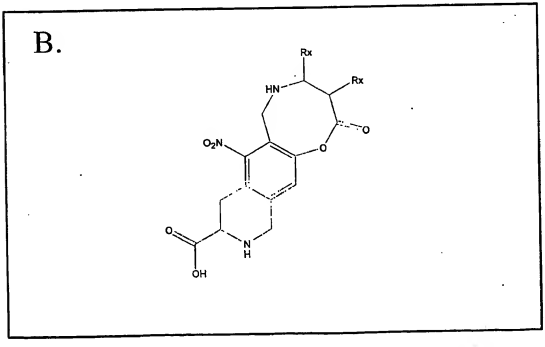


Fig. 22. Templated polymers.

- alpha-, beta-, gamma-, and omega-peptides
- mono-, di- and tri-substituted peptides
- L- and D-form peptides
- cyclohexane- and cyclopentane-backbone modified beta-peptides
- vinylogous polypeptides
- glycopolypeptides
- polyamides
- vinylogous sulfonamide peptide
- Polysulfonamide
- conjugated peptide (i.e., having prosthetic groups)
- Polyesters
- Polysaccharides
- Polycarbamates
- Polycarbonates
- Polyureas
- poly-peptidylphosphonates
- Azatides
- peptoids (oligo N-substituted glycines)
- Polyethers
- ethoxyformacetal oligomers
- poly-thioethers
- polyethylene glycols (PEG)
- Polyethylenes
- Polydisulfides
- polyarylene sulfides
- Polynucleotides
- PNAs
- LNAs
- Morpholinos
- oligo pyrrolinone
- polyoximes
- Polyimines
- Polyethyleneimine
- Polyacetates
- Polystyrenes
- Polyacetylene
- Polyvinyl
- Lipids
- Phospholipids
- Glycolipids
- polycycles (aliphatic)
- polycycles (aromatic)
- polyheterocycles
- Proteoglycan
- Polysiloxanes
- Polyisocyanides
- Polyisocyanates
- Polymethacrylates

Fig. 23. Precursors - examples.

- N-carboxyanhydrides (NCA)
- N-thiocarboxyanhydrides (NTA)
- Amines
- Carboxylic acids
- Ketones
- Aldehydes
- Hydroxyls
- Thiols
- Esters
- Thioesters
- conjugated system of double bonds
- Alkyl halides
- Hydrazines
- N-hydroxysuccinimide esters
- Epoxides
- Haloacetyls
- UDP-activated saccharides
- Sulfides
- Cyanates
- Carbonylimidazole
- Thiazinanones
- Phosphines
- Hydroxylamines
- Sulfonates
- Activated nucleotides
- Vinylchloride
- Alkenes, quinones

Fig. 24. Functional groups – examples.

- Hydroxyls
- Primary, secondary, tertiary amines
- Carboxylic acids
- Phosphates, phosphonates
- Sulfonates, sulfonamides
- Amides
- Carbamates
- Carbonates
- Ureas
- Alkanes, Alkenes, Alkynes
- Anhydrides
- Ketones
- Aldehydes
- Nitatrates, nitrites
- Imines
- Phenyl and other aromatic groups
- Pyridines, pyrimidines, purines, indole, imidazole, and heterocyclic bases
- Heterocycles
- polycycles
- Flavins
- Halides
- Metals
- Chelates
- Mechanism based inhibitors
- Small molecule catalysts
- Dextrins, saccharides
- Fluorescein, Rhodamine and other fluorophores
- Polyketides, peptides, various polymers
- Enzymes and ribozymes and other biological catalysts
- Functional groups for post-polymerization/post activation coupling of functional groups
- Drugs, e.g., taxol moiety, acyclovir moiety, "natural products"
- Supramolecular structures, e.g. nanoclusters
- Lipids
- Oligonucleotides, oligonucleotide analogs (e.g., PNA, LNA, morpholinos)

Fig. 25. Polymers and the functional entities required to make them.

A.

Polymer	Functional Entity (reactive groups)	Linking molecule	Catalyst/reagent	General Figure	Specific Figure
polycyclic compound	di-coumarin		light	Fig. 11	Fig. 11, ex. 1
polyester	alcohol, carboxylic acid		carbodiimide	Fig. 12, Fig. 21	
polyester	hydroxyl, thioester			Fig. 14	
polyurea	di-amine	carbonyldiimidazole		Fig. 15	Fig 15, ex. 3
polyacetate	halogen, carboxylic acid		base	Fig. 12, Fig. 21	
polyacetate	alcohol, carboxylic acid		EDC or other carbodiimide	Fig. 12, Fig. 21	
polycarbamate	alcohol, isocyanate			Fig. 12, Fig. 21	
polycarbonate	diol	carbonyldiimidazole		Fig. 15	
peptoid	secondary amine, α-haloacetyl			Fig. 12, Fig. 21	
	primary amine, α-haloacetyl		alkylating agent	Fig. 12, Fig. 21	
glycogen	UDP-glucose		glycogen synthetase	Fig. 12, Fig. 21	
polysaccharide	UDP-activated saccharides		polysaccharide synthetases	Fig. 12, Fig. 21	
polysaccharide	glucosyl sulphide/sulfoxide activation system (Kahne glucosylation)		Kahne conditions	Fig. 12, Fig. 21	
polyamide	amine, N-hydroxysuccinimide ester			Fig. 12, Fig. 21	
polyamide	amine, carboxylic acid		carbodiimide	Fig. 12, Fig. 21	

Fig. 25, continued

Polymers and the functional entities required to make them.

B.

Polymer	Functional Entity (reactive groups)	Linking molecule	Catalyst/reagent	General Figure	Specific Figure
polyamide	di-amine	di-carboxylic acid	carbodiimide	Fig. 15	Fig. 15, ex. 2
polyamide	di-carboxylic acid	di-amine	carbodiimide	Fig. 15	
polyamide	amine, carboxylic acid	amine, carboxylic acid	carbodiimide	Fig. 16	
α-polypeptide	carboxyanhydride (5-membered ring)			Fig. 18	
β-polypeptide	carboxyanhydride (6 membered ring)			Fig. 18	Fig. 18, ex.1
γ-polypeptide	carboxyanhydride (7-membered ring)			Fig. 18	
α-polypeptide	2,2-diphenylthiazinanone (5-membered ring)			Fig. 18	
β-polypeptide	2,2-diphenylthiazinanone (6-membered ring)			Fig. 18	Fig. 18, ex.2
γ-polypeptide	2,2-diphenylthiazinanone (7-membered ring)			Fig. 18	
α-polypeptide	amine, thioester			Fig. 14	
β-polypeptide	amine, thioester			Fig. 14	Fig. 14, ex.1
γ-polypeptide	amine, thioester			Fig. 14	
ω-polypeptide	amine, thioester			Fig. 14	
polysulfonamide	amine, sulfonic acid		carbodiimide	Fig. 12, Fig. 21	
polyphosphonate	di-alcohol	activated phosphonate		Fig. 15	
polyphosphonate	di-alcohol	activated alkylphosphine	oxidating reagent, e.g. tert-butylhydroperoxide	Fig. 15	
polyphosphate	di-alcohol	diaminoalkoxy-phosphine	oxidating reagent, e.g. tertbutyl-hydroperoxide	Fig. 15	
polyphosphodiester	diol	diaminophosphine	oxidant (ButOOH)	Fig. 15	Fig. 15, ex. 5
polyphosphodiester	diaminophosphine	diol	oxidant (ButOOH)	Fig. 15	Fig. 15, ex. 6

Fig. 25, continued
Polymers and the functional entities required to make them.

C.

Polymer	Functional Entity (reactive groups)	Linking molecule	Catalyst/reagent	General Figure	Specific Figure
polyurethane	diamine	diisocyanate		Fig. 15	
polyether	epoxide			Fig. 18	Fig. 18, ex. 3
polythioether	thioepoxide			Fig. 18	
polydisulfide	thiol, thiol		oxidant	Fig. 11	
polyoxime	aldehyde, hydroxylamine			Fig. 12, Fig. 21	
polyimine	aldehyde, amine			Fig. 12, Fig. 21	
polyimine	aldehyde, amine			Fig. 15	Fig. 15, ex. 1
polynucleotides	nucleoside-5'-phosphoro-2-methylimidazolides			Fig. 12, Fig. 21	
polyamine	amine, alkyl sulfonate			Fig. 14	Fig. 14, ex.2
alkane	alkene			Fig. 17	Fig. 17, ex. 1
alkane	alkene			Fig. 17	Fig. 17, ex.2
polycycloalkane	di-diene	di-alkene (benzoquinone)		Fig. 15	Fig. 15, ex. 7
polyvinyl	vinylchloride unit			Fig. 17	
polystyrene	styrene-unit		radical initiator, AIBN	Fig. 17	
polyethylene	ethylene unit			Fig. 17	Fig. 17, ex. 1

Fig. 26

Protocol for chemical charging of specific tRNAs

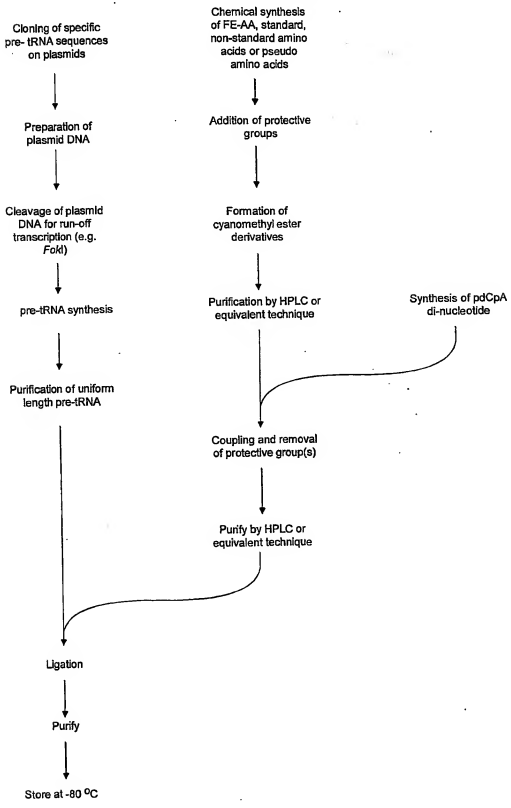


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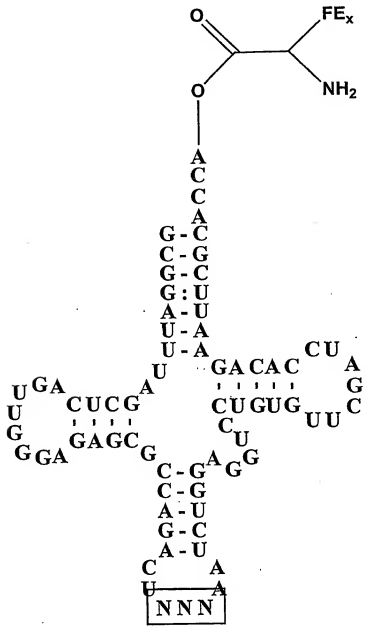
Fig. 27A

An example of a general structure for a set of building blocks.



Variable sequence (i.e. anticodon)

Fig. 27B

Examples of anticodon sequences and their corresponding functional entities

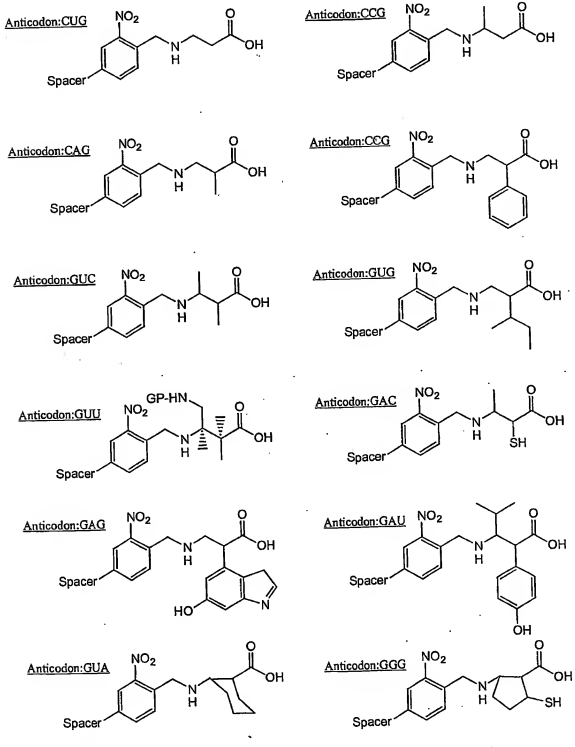


Fig. 28

Bond formation and linker cleavage

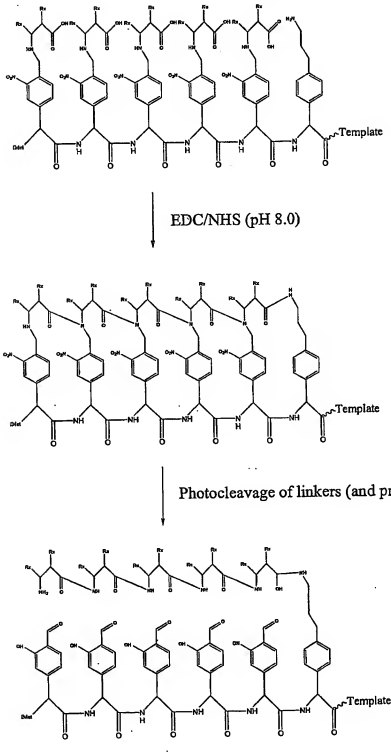


Fig. 29 Pairs of reactive groups X, Y and the resulting bond XY.

Nucleophilic substitution reaction

Fig. 29, continued

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Fig. 29, continued

Cycloaddition to multiple bounds

Fig. 29, continued

Addition to carbon-hetero multiple bonds